ヘビイチゴ，ヤブヘビイチゴ，及びその雑種の比較解剖学

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Naohiro NARUHASHI* and Hidetomo ISHIZU* : Comparative Anatomy of *Duchesnea chrysantha, D. indica* and Their Hybrids (Rosaceae)

鳴橋直弘*・石須秀知*：ヘビイチゴ，ヤブヘビイチゴ，及びその雑種の比較解剖学

Abstract

The anatomical characters of three taxa of *Duchesnea, D. chrysantha* (diploid), *D. indica* (dodecaploid), *D.*× *harakurosawae* (heptaploid and octoploid) were compared. Leaf, runner, rhizome, root, flower and fruit are examined histologically. Anatomical differences between *D. chrysantha* and *D. indica* are quantitative in all the cases. The natural hybrid, *D.* ×*harakurosawae,* is intermediate in anatomical characteristics between the parental species.

Key Words: Anatomy—*Duchesnea*—Hybrid—Morphology

Introduction

Duchesnea (Rosaceae) consists of two species; *D. chrysantha* (ZOLL. et MOR.) MIQ., diploid and *D. indica* (ANDR.) FOCKE, dodecaploid (HARA and KUROSAWA, 1959). The natural hybrid is named *D.* × *harakurosawae* (NARUHASHI and SUGIMOTO, 1986), which is consisted of two cytotypes: heptaploid (7 X) and octoploid (8X). The two types of hybrid are found frequently in Chubu District of Honshu (NARUHASHI et al., 1986: NARUHASHI and TAKANO, 1987: NARUHASHI et al., unpublished).

Cytological and cytogenetical studies (NARUHASHI and IWATSUBO, 1991a, 1991b) show that no common genom set exists in *D. chrysantha* and *D. indica,* and that 7X hybrid have each of half a mixture of a set of chromosomes of the two species, and 8X hybrid have a full set of chromosomes of *D. chrysantha* with half a set of *D. indica.*

Under the recent outer morphological investigation of *Duchesnea* showed that the hybrids have intermediate features in many characters, and furthermore 7X hybrid is closer to *D. indica* than 8X hybrid (SUGIMOTO et al., 1991). This morphological result agreed to cytological and cytogenetical data.

Though cytological differences were clarified, the two species had small amount of the outer morphological differences (SUGIMOTO et al., 1991).

There has not been any anatomical study on the genus. The present investigation was carried out to elucidate the differentiation of the inner morpholgy in the four cytotypes.

Materials and methods

Three taxa of *Duchesnea*; *D. chrysantha, D. indica,* and two cytotypes of *D.* × *harakurosawae* were examined. These plants are cultivated in the almost same condition in the Botanic Garden of Toyama University and their original localities are as follows:

D. chrysantha: Chiharasaki, Toyama City, Toyama Pref.

D. indica: Chiharasaki, Toyama City, Toyama Pref.

D. × *harakurosawae* 7X: Gofuku, Toyama City, Toyama Pref.

D. × *harakurosawae* 8X: Kurehayama, Toyama City, Toyama Pref.

Plants of natural populations in Toyama City were also used for confirmation of some results. Voucher specimens have been deposited at the Herbarium of Department of Botany, Faculty of Science, Toyama University.

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Of the all taxa in question, leaf, stem (runner and rhizome), root, flower and fruit (false fruit) were observed. Most of these organs were examined fresh and a few were prefixed in FAA. Transverse sections were hand-cut (approx. 20–30 μm thick) using razor blade. Five sections from different individuals on each cytotype were made and either stained with hematoxylin or unstained, were examined microscopically.

Results

The results of anatomical examination on the leaf, runner, rhizome, root, flower and fruit are described below.

a) Leaf

Duchesnea has ternate leaves with long petiol and stipules. Lamina consists of upper epidermis, palisade tissue, spongy tissue and lower epidermis and vascular bundles within it. *Duchesnea chrysantha* has one layer palisade tissue or rarely obscurely two layer palisade tissue. Two layer palisade tissue, however, is obviously observed in *D. indica* (Fig. 1). Those of 7X and 8X hybrid are almost the same as shown in *D. indica.*

The vascular system of petiole is the same among the present taxa (Fig. 2). That is, three major bundles, one central vascular bundle and two lateral vascular bundles run into the basal part of petiole from stem, and the two lateral vascular bundles divide into two bundles respectively after the separation of thin bundles running to stipule. Inner one of the two bundles from the lateral vascular bundles joins to the central vascular bundle and the two laterals divide again into two bundles respectively at the upper part of petiole. The outer one of the two bundles run into the lateral leaflets. While the inner one goes to the center and join to the central vascular bundle at the somewhat broaden portion of petiole. At this level, the central vascular bundle divides into three bundles running into the leaflets as the main veins.

b) Runner

In spring *Duchesnea* extend a few runners bearing a few flowers which develop on each node as a solitary inflorescence.

As shown in the transverse sections (Fig. 3), a runner in *Duchesnea* consists of epidermis, a few layered cortex, endodermis, cylindrical sclerenchyma, vascular bundle and pith. On the observation of the second internode of the runner from apex, the development of the sclerenchyma surrounding stele is weak in *D. chrysantha* as compared with *D. indica.* On the other point of view, however, no remarkable differences are detected. There are many simple starch grains stored in both the parenchyma of cortex and pith. The starch grains in cortex are ralatively small size and show small difference among taxa. Those in pith, however, are conspicuously large and densely distributed in *D. chrysantha,* while those of *D. indica* are small and sparsely dis-

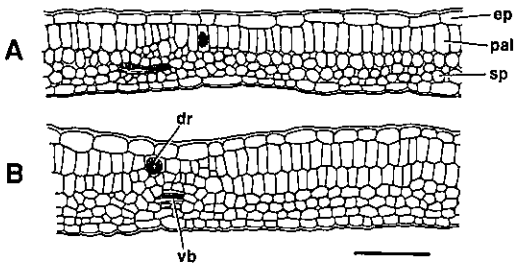

Fig. 1. Cross section of lamina.
A: *D. chrysantha*; B: *D. indica.* dr; druse; ep: epidermis; pal: palisade tissue; sp: spongy tissue; vb: vascular bundle. (bar: 100μm)

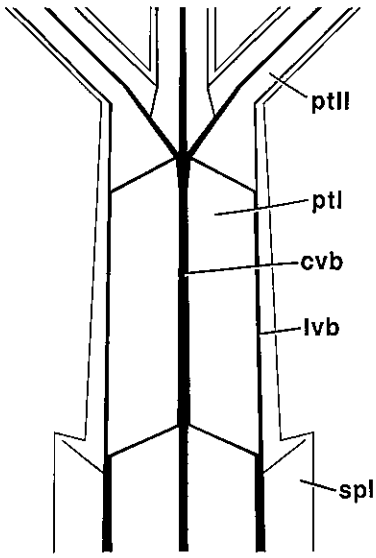

Fig. 2. Vascular system of petiole.
cvb: central vascular bundle; lvb: lateral vascular bundle; ptI: petiole; ptII: petiolule; spI: stipule.

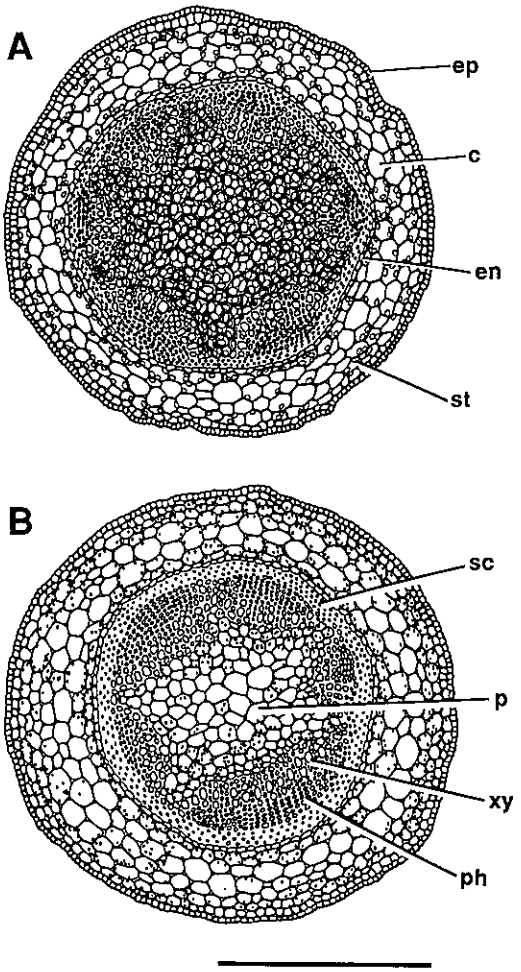

Fig. 3. Cross section of runner.
A: *D. chrysantha*; B: *D. indica*.
c: cortex; en: endodermis; ep: epidermis; p: pith; ph: phloem; sc: sclerenchyma; st: starch grain; xy: xylem. (bar: 0.5mm)

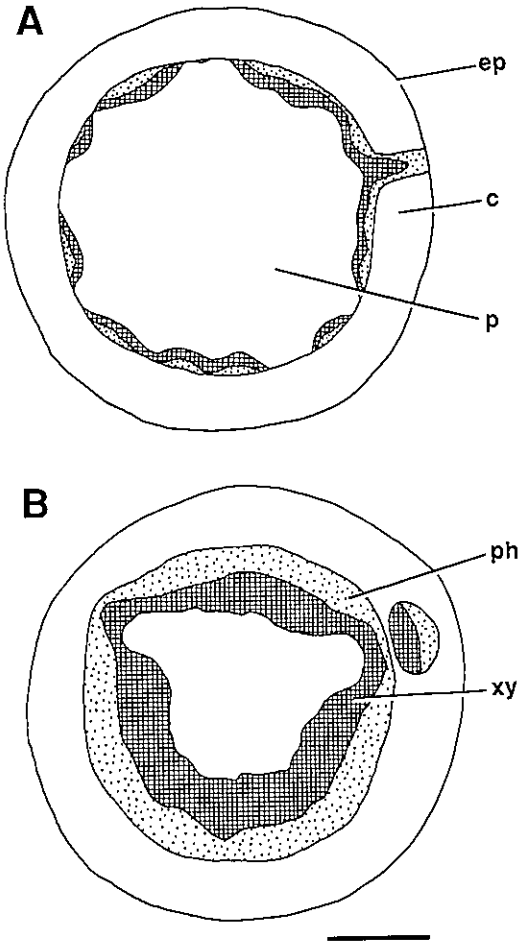

Fig. 4. Cross section of rhizome.
A: *D. chrysantha*; B: *D. indica*.
c: cortex; ep: epidermis; p: pith; ph: phloem; xy: xylem. (bar: 1mm)

tributed (Fig. 3). Starch grains of 7X and 8X hybrid are intermediate in size and density between the parental species.

c) Rhizome

Swollen rhizomes of *D. chrysantha* in early summer and autumn are observed. The swollen rhizomes of *D. indica*, however, are not observed throughout the year. The swollen rhizome of *D. chrysantha* shows weakly developed vascular bundle and well-developed parenchyma of pith, while that of *D. indica* shows well-developed vascular bundle and narrow pith (Figs. 4 and 5). The histological characteristics of the rizomes in *D.* × *harakurosawae* 7X and 8X shows the same as that of *D. indica*.

Many large starch grains are stored in the pith in *D. chrysantha*, while starch grains are less and smaller in *D. indica* (Fig. 6). On the other hand, many druses of calcium oxalate occur in the pith and cortex in *D. indica*, but rarely in *D. chrysantha* (Figs. 5 and 6). In *D.* × *harakurosawae* 7X and 8X, both of starch grains and druses are observed and the starch grains are fewer and smaller than those of *D. chrysantha*.

d) Root

Duchesnea has normal roots sprouting from rhizome of stock and adventitious roots sprouting from each nodes of runner. The transverse sec-

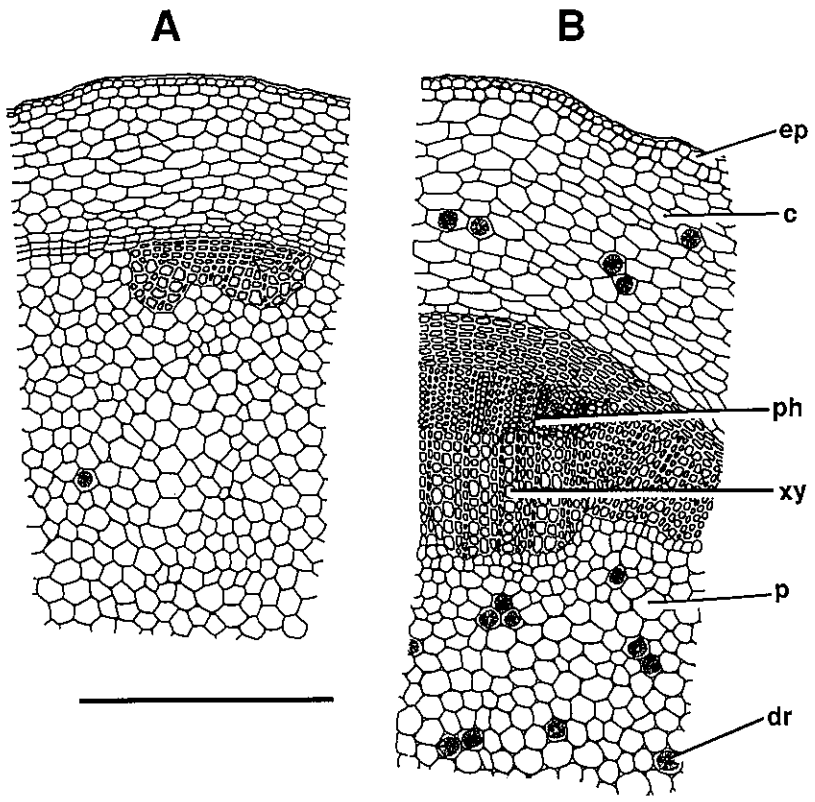

Fig. 5. Detailed drawing of rhizome.
A : *D. chrysantha* ; B : *D. indica.*
c : cortex ; dr : druse ; ep : epidermis ; p : pith ; ph : phloem ; vb : vascular bundle ; xy : xylem. (bar : 0.5mm)

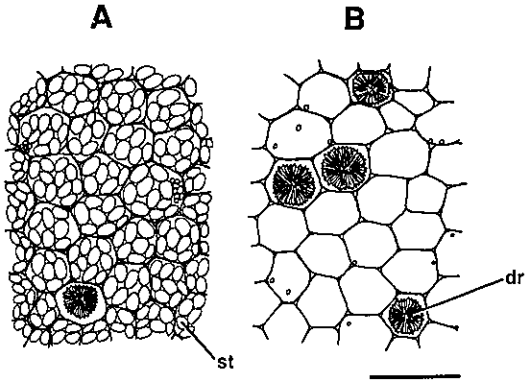

Fig. 6. Part of pith of rhizome.
A : *D. chrysantha* ; B : *D. indica.*
dr : druse ; st : starch grain. (bar : 100μm)

tions of root are shown in Fig. 7 demonstrating epidermis, cortex, endodermis, pericycle, phloem, xylem, and rather sclerenchymatous pith from outer to inner part. In the cortex intercellular spaces are observed. Epidermis and some part of cortex in older root often peels off or disappers. There is no difference among the taxa in such fundamental composition of the root.

e) Flower

The flower of *Duchesnea* is 5-merous, namely 5 petals, 5 calyces, 5 epicalyces, 20 stamens and many pistils on the receptacle. Calyx tube is almost flat and nectar is located inside of almost the middle part of calyx tube.

A common vascular system of flowers in all taxa was observed. Namely, there are 20 vascular bundles at the basal part of flower. The alternate 10 of them spread radially and run into calyx tube and 10 remainders run into ovaries through the receptacle (ov). The radiate vescular bundles

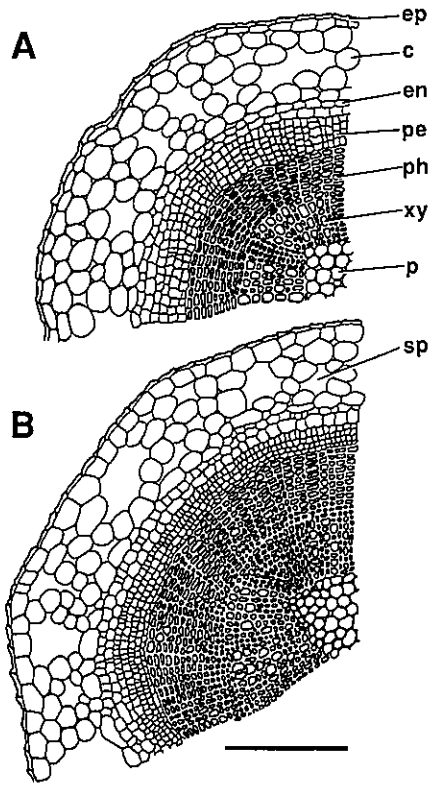

Fig. 7. Cross section of root.
A: *D. chrysantha*; B: *D. indica*.
c: cortex; en: endodermis; ep: epidermis; p: pith; pe: pericycle; ph: phloem; sp: intercellular space; xy: xylem. (bar: 200μm)

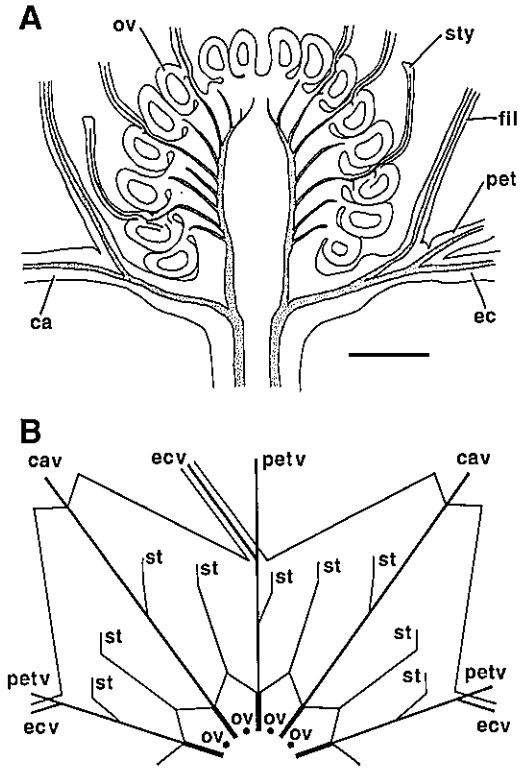

Fig. 8. Flower of *Duchesnea chrysantha*.
A: Longitudinal section of flower; B: Vascular system of flower.
ca: calyx; ec: epicalyx; fil: filament; ov: ovary; pet: petal; sty: style; cax: vascular of calyx; ecv: vascular of epicalyx; ov: vascular of ovary; petv: vascular of petal; st: vascular of stamen. (bar: 1 mm)

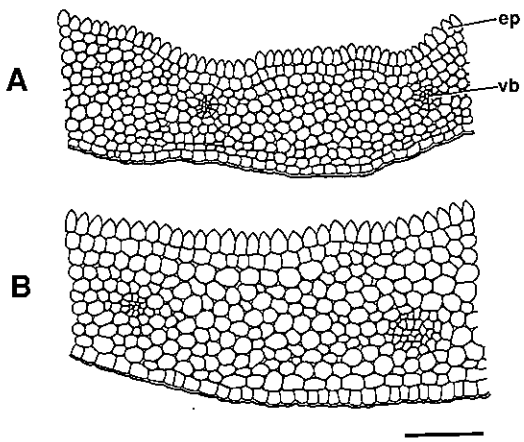

Fig. 9. Cross section of petal.
A: *D. chrysantha*; B: *D. indica*.
ep: epidermis; vb: vascular bundle. (bar: 100μm)

divide into the vascular supply of stamens (st) and others. Others divide again at the margin of calyx tube and alternate 5 of them run into epicalyx (ecv), petals (petv) and stamens (st), and 5 of remains into calyx (cav) and stamens (st). (see, Fig. 8).

Transverse section of petals of two species are shown in Fig. 9. From the upper surface to the lower surface, papillae on the upper surface, parenchyma with vascular bundle, and epidermis of somewhat thick walled cells on the lower surface are observed. There is no structural difference in petal anatomy between the two species.

7X and 8X hybrids show the same histological characteristics.

Figure 10 illustrates the transverse section of calyces of the two species. There is a cell layer like palisade under the epidermis of adaxial surface and parenchyma occupies innermost part of

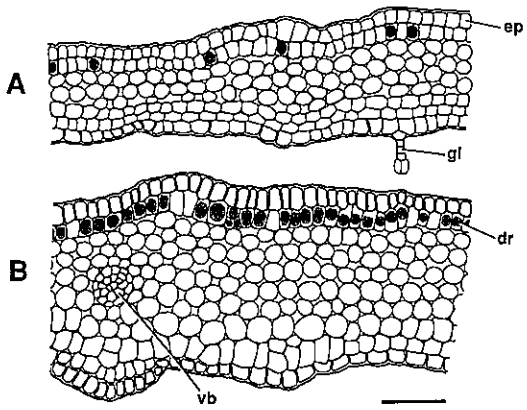

Fig. 10. Cross section of calyx.
A: *D. chrysantha*; B: *D. indica*.
dr: druse; ep: epidermis; gl: glandular hair; vb: vascular bundle. (bar: 100μm)

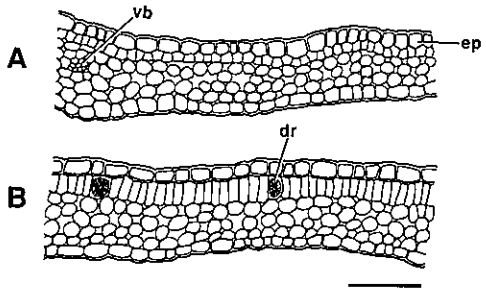

Fig. 11. Cross section of epicalyx.
A: *D. chrysantha*; B: *D. indica*.
dr: druce; ep: epidermis; vb: vascular bundle. (bar: 100μm)

the calyces. Many calcium oxalate crystals in the palisade like tissue are found in *D. indica* and *D.* × *harakurosawae* 7X and 8X, but rarely in *D. chrysantha*.

Inner morphological structures of epicalyx of the two species are shown in Fig. 11, in which the first cell layer under the epidermis of adaxial surface in *D. indica* looks like a palisade tissue. On the other hand, *D. chrysantha* does not have a cell layer like a palisade tissue. As for this character, intermediate condition is observed in *D.* × *harakurosawae* 7X and 8X.

The anatomy peduncles is comparable to that of the runner in a fundamental composition.

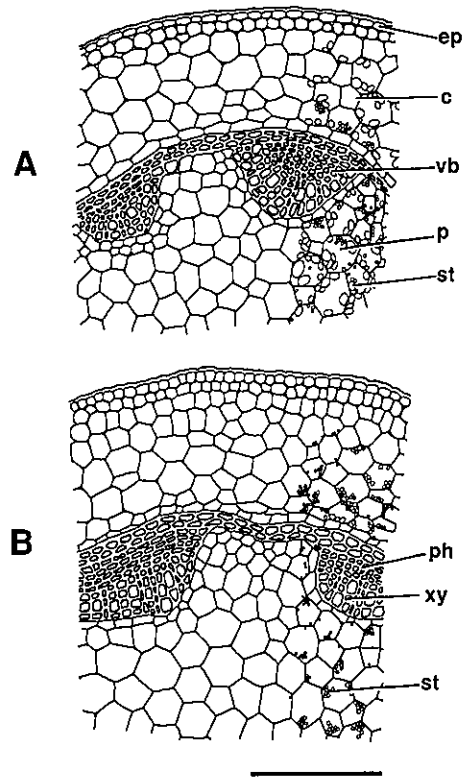

Fig. 12. Cross section of peduncle.
A: *D. chrysantha*; B: *D. indica*.
c: cortex; ep: epidermis; p: pith; ph: phloem; st: starch grain; vb: vascular bundle; xy: xylem. (bar: 200μm)

Namely, epidermis, cortex, sclerenchyma, vascular bundle and pith are recognized also in the peduncle (Fig. 12). However, in some respects peduncles differ from runners. Peduncles have weakly developed sclerenchyma which make no circle at the upper portion. The peduncle bundles running into a flower are constantly 10 in number though vasculization at the basal part of the peduncle does not clearly show its regularity. Starch grains in pith and cortex are observed, but they are not many as shown in those of runner. The starch grains of *D. chrysantha* are about 10 μm in diameter and those of *D. indica* and *D.* × *harakurosawae* 7X and 8X are smaller.

f) Fruit

Duchesnea produces a false fruit by means of enlargement of receptacle after flowering. But in *D.* × *harakurosawae* 7X and 8X, receptacles show no enlargement and no fruit is produced.

Longitudinal section of an achen is observed

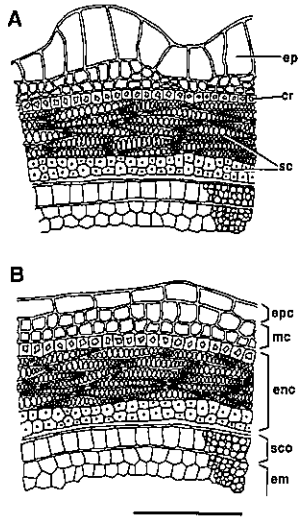

Fig. 13. Longitudinal section of achene.
A: *D. chrysantha*; B: *D. indica*.
cr: crystal; em: embryo; enc: endocarp; ep: epidermis; epc: epicarp; mc: mesocarp; sc: sclereid; sco: seed coat. (bar: 100μm)

(Fig. 13). Rugose-tubercled achenes in *D. chrysantha* result from irregular enlargement of the cells in the epicarp. While in *D. indica* the surface of achene is smooth resulting from cells of epicarp with almost the same size. Mesocarp and endocarp in both taxa are the same, namely mesocarp consist of 2 to 3 somewhat thick-walled cell layer with plastids, and endocarp consist of a single cell layer containing rhombohedral crystals, and thick layer of schlerenchyma consisting of two layers; long spindle-shaped schlerenchyma cells with so-called fiber-septate standing in a longitudinal direction of an achene and the same standing in a tangential direction of an achene. The seed coat consists of only two layers of cells, the upper one of which is thin in a radial direction, and the lower one is parenchymatous and containing densely ergastic substances stored for nutrition as in embryo.

Discussion

On the basis of the results given above, the following histological differences of some organs were observed among the taxa in question.

The surface of achenes of *D. chysantha* is rugose-tubercled, while that of *D. indica* is smooth. The presence or absence of the rugose -tubercles on the achene surface was often used as a key character distinguishing the two species from each other (HARA and KUROSAWA, 1959; OHWI, 1965). The present anatomical study makes it clear that the presence of rugose-tuberclation is caused by the irregular enlargement of cells of the outermost layer of the fruit-wall, and that no fundamental difference is recognized between the presence and absence of rugose-tuberculation. Actually slightly rogose-tuberculation is observed in *D. chrysantha*.

In the materials used in this study, starch grains of *D. chrysantha* are large and accumulated much, whereas those of *D. indica* are small both in size and quantity. This difference, *i.e.*, the difference in size and amount of starch grains result from the difference of the physiological and ecological characters on the dry matter economy of two species. The two species is not characterized by the size and quantity of starch grains, because the starch grains are considered to change in size and quantity by environmental condition, especially by seasonal factor.

Cylindrical sclerenchyma is well developed in the runner of *D. chrysantha* and *D. indica*, and also observed in the runner of *Fragaria* and *Potentilla* (unpublished). This character may be understood from adaptation to the external pressure of bending, pulling, *etc.* The sclenrenchyma of both species of *Duchesnea* are essentially the same except for the difference in degree of development.

The rhizomes of *D. indica* are apparently constant in outer morphology throughout the year, while those of *D. chrysantha* are swollen in early summer and autumn and there are some plant show remarkable swollen rhizome in winter time. This functional difference of rhizome in *Duchesnea* is considered, *i.e.*, the rhizome of *D. chrysantha* develop the function for stored organ, while the rhizome of *D. indica* develop the mechanical supporting function. The former contains much starch grains and the latter contains less in the rhizome of winter time. The difference of the storing starch grains is explained as a high dry matter allocation to the runner and rhizome in *D. chrysantha* than *D. indica* (SUGIMOTO and NARUHASHI, 1981). Though the remarkable outer morphological feature of rhizome in two species is found, presence of special tissue (s) is not recognized in even one of two species. Therefore the

difference of the rhizome from the outer morphology is considered as the difference of size and amount of the same tissue.

Duchesnea chysantha and *D. indica* are very close in taxonomic relationships (WOLF, 1908; KITAMURA and MURATA, 1961; KALKMAN, 1968), and have the almost same habitat (KUME et al., 1987) and life cycle (SUGIMOTO and NARUHASHI, 1981), and show small differences on outer morphological characters (SUGIMOTO et al., 1991). On the anatomy, the histological differences of two species are shown as a quantitative one such as a different size of tissue without different kinds and arrangement of tissues.

Though cytological studies (NARUHASHI and IWATSUBO, 1991a, 1991b) make clear that no common genom between two species exist, the two species are similar in inner morphology as well as outer morphology.

Duchesnea × *harakurosawae* 7X and 8X are similar to each other in almost all anatomical characters. Many characters such as size and amount of starch grains, formation of sclerenchyma in runner and shape of cells of palisade in epicalyx, *etc.* are intermediate between the parental species. In some characters, however, such as structure of rhizome, palisade tissue of lamina and size and amount of druse, *etc.* they are very similar to *D. indica.*

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摘　要

バラ科ヘビイチゴ属の2倍体(ヘビイチゴ)，7倍体と8倍体（種間雑種），及び12倍体（ヤブヘビイチゴ）の内部形態を詳細に比較検討した。

細胞学的な研究から，ヘビイチゴとヤブヘビイチゴの核ゲノムの分化が著しいにもかかわらず，両種の観察された解剖学的な差はほとんどの形質において量的な差であり，組織学的な分化の低いことがわかった。両種の内部形態の組織の種類や配列の共通性は，外部形態と同様に，それらの生育地や生活史の類似と関係するものと考えられる。なお，雑種は，多くの形質において両親の中間の形態を示した。

最近の分類学者は，ヘビイチゴとヤブヘビイチゴの検索表に痩果の表面のしわの有無を使用しているが，これは果実の最外層の細胞の不規則な肥大に起因し，量的な差と考えられ，検索表に使用するほどのギャップのある形質と見なされないので，検索形質に用いない方がよいと思われる。

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